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The influence of CYP enzymes and ABCB1 on treatment outcomes in schizophrenia: association of CYP1A2 activity with adverse effects

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Abstract

Aim: Genetic variants on metabolic and transport enzymes are good candidates to explain inter-individual differences in response to antipsychotics. The aim of this study is to evaluate and compare the influence of the *CYP2D6*, *CYP2C19*, *CYP1A2* and *ABCB1* variants on plasma levels, treatment response and side effects of antipsychotics.

Methods: Twenty polymorphisms in selected genes were genotyped in 318 patients diagnosed with schizophrenia, schizoaffective or delusional disorder treated with antipsychotics (clozapine, olanzapine, paliperidone, risperidone, aripiprazole and quetiapine). Plasma drug levels were determined after 6 weeks of treatment. The Positive and Negative Symptoms Scale (PANSS) and UKU scale of side effects were recorded at baseline and after 12 weeks



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of treatment. The effect of gene variants on plasma drug levels, treatment response and adverse effects were examined by multinomial regression.

Results: *CYP1A2* was found to be associated with psychic side effects ($P = 0.02$), with variants predicting higher enzyme activity associated with lower adverse effects, and was the strongest predictor for this adverse effect of all the studied factors. Functional variants in *CYP* genes were associated with plasma level differences, with higher activity variants associated with lower plasma levels. No association with improvement of the condition, as measured by the PANSS score, was found in this study.

Conclusion: The results suggest that increased *CYP1A2* activity protects against psychic side effects. Few studies have evaluated the impact of genetic factors on treatment response or side effects, and only in relation to a selection of adverse reactions. These results are a step towards better understanding of the factors behind the different aspects of clinical outcomes, such as various adverse effects.

Keywords: Pharmacogenetics, antipsychotics, *CYP1A2*, *CYP2C19*, *CYP2D6*, *ABCB1*, drug response, adverse drug reactions

INTRODUCTION

Antipsychotic drugs are the mainstay of treatment for severe mental disorders such as schizophrenia and bipolar disorders. The treatment response to antipsychotic medications has wide inter-individual variability. About 30%-50% do not respond adequately and 50% develop side effects^[1]. Pharmacokinetics are expected to play an important role in drug response. The effective antipsychotic dose varies among patients, and drug response is highly related to plasma levels, as is drug safety^[2]. Plasma levels are a direct consequence of the administered dose, but variations on pharmacokinetics, namely drug absorption and metabolism, introduce an element of uncertainty in plasma levels. At the same time, plasma levels and all processes capable of affecting them may modify the dose-dependent fraction of the drug's response. These processes include transport across biological membranes, such as absorption and excretion, and metabolism. Individuals with greater metabolism may require higher doses, while those with lower metabolic rates might be more prone to the drugs' adverse effects and need dose reduction^[3-7]. With all these variables potential predictors of drug response, which of them, or combination of, is the best predictor of clinical endpoints, if at all.

Genetic variants are known to influence both drug transport and metabolism. Variants in certain genes [i.e., members of the cytochrome (CYP) P450 superfamily, or CYPs] have attracted attention because of their potential to influence treatment response. The CYP superfamily of enzymes is responsible for the metabolism of most drugs used in medical practice, including psychiatric drugs. The activity of the *CYP* enzymes, as predicted by the allelic variants of the genes encoding for them, can be classified into one of five groups: poor, intermediate, normal, rapid or ultrarapid metabolizers. Most psychiatric drugs are metabolized by the enzymes *CYP2D6*, *CYP1A2* and *CYP2C19*. Another potentially important gene is *ABCB1*, which encodes the transport protein MDR1. This protein is expressed in the luminal membrane of enterocytes and it can influence the absorption and bioavailability of substrate drugs. *ABCB1* is also expressed in the blood-brain barrier and can affect penetration into the central nervous system^[8,9]. Although *CYP* genetic variants have been widely investigated in relation to mental disorders and their treatment, the contribution of *ABCB1* to treatment response and its possible interactions with *CYP* functional variants is scarcely known. The identification of factors with greater influence on clinical doses and response to psychotropic drugs may have an important effect on the improvement of treatment in psychiatry. *CYP* enzymes can have genetic variation ranging from loss-of-function alleles to full gene

duplications, which can have drastic effects on enzymatic activity. The consequences of these variants on the plasma levels of drugs have been extensively reported^[3,10-12]. The contribution of *ABCB1* to plasma levels has been investigated, although there is little evidence that this transporter plays a relevant role^[6,12,13]. However, there are studies that have reported an association between *ABCB1* variants and treatment response, albeit in moderate sample sizes or healthy volunteers^[14]. The number of studies focusing on pharmacokinetic parameters vastly outnumbers publications evaluating treatment response and side effects. Of these, results on clinical outcomes are unclear. For *CYP2D6*, there are negative reports^[15], although it has also been reported that improvement of the condition is affected by the metabolizing phenotype^[7,16]. The situation is similar for *ABCB1*, with some studies having found association^[17,18] with antipsychotic responses while others have not^[19]. There have also been reports of association with genetic variation in *CYPs* and *ABCB1* with adverse effects^[20-23]. These studies focus on particular adverse effects, such as QT interval prolongation, dyskinesias and prolactinemia. However, no study has evaluated the impact on a wide spectrum of possible adverse effects.

The objective of this study is to evaluate the contribution of genetic variants in *CYPs*, and *ABCB1* genes on antipsychotic drug doses, treatment response and induced side effects, in order to select the factors most predictive of response. To achieve this objective, data regarding efficacy, side-effects, plasma levels and *CYPs* and *ABCB1* genetic variants were collected in a sample of patients treated with antipsychotics. These patients were participants of a previous study^[24] investigating the efficiency of a pharmacogenetic algorithm using the genotype of different *CYPs*, which concluded that the inclusion of pharmacogenetic information was beneficial for treatment. However, plasma levels were not investigated other than for assessing adherence, and neither was drug transport or in-depth analysis of adverse effects.

METHODS

Sample

Three hundred and eighteen patients from a previous work^[24] were included in the study. The gender distribution was 55% male and 45% female. Mean age was 47 years old. These patients all had a diagnosis of schizophrenia, schizoaffective or delusional disorder. The most commonly used drug was clozapine (135 patients) followed by olanzapine (41 patients), paliperidone (39 patients), risperidone (38 patients), aripiprazole (21 patients), and quetiapine (18 patients); the remaining patients used other drugs. All patients used a single antipsychotic for the duration of the study. One hundred and thirty of these patients received a pharmacogenetic intervention consisting of adjustment of their antipsychotic drug dose according to their *CYP* genetic profile when required, while the rest received standard treatment. This cohort is described in Table 1.

Biochemical parameters

The plasma concentrations of both the antipsychotic and its main metabolite were determined at 6 weeks after starting treatment, when steady state was achieved. Since treatment doses were not modified after reaching steady state, plasma levels were assumed to be stable for the remainder of the treatment. The plasma concentration of parent antipsychotic drugs, metabolites and active moiety were measured by a validated high-performance liquid chromatography (HPLC) method, using ultraviolet visible diode array detection and solid-phase extraction on cyano cartridges. Compounds were separated on a C8 reversed-phase column with a gradient of acetonitrile and phosphate buffer 50 mM, pH 3.8 and detected at 210 and 278 nm. To assure high precision and accuracy of the method, an internal standard was included in the sample preparation and quantitative evaluation. The within- and between day precision expressed as coefficient of variation (CV)% were < 10%. The quality of analyses was subject to a program of internal quality control and external quality assessment^[25].

Table 1. General information about the study sample

Age	Mean (years)	SD (%)
	43.9	13.8%
Gender		Percent
Men	175	55%
Women	143	45%
Diagnosis		Percent
Schizophrenia	243	76.4%
Major depression	3	0.9%
Brief psychosis	1	0.4%
Delusional disorder	55	17.3%
Schizoaffective disorder	16	5%
Treatment		Percent
Clozapine	141	44.3%
Olanzapine	43	13.5%
Paliperidone	41	12.9%
Risperidone	40	12.6%
Aripiprazole	22	6.9%
Quetiapine	19	5.9%
Ziprasidone	3	0.9%
Haloperidol	2	0.6%
Asenapine	2	0.6%
Levomepromazine	2	0.6%
Trifluoperazine	2	0.6%
Perfenazine	1	0.3%
Pimozide	1	0.3%
Altered PGX		Percent
Yes	243	76.4%
No	75	23.6%

SD: standard deviation; Altered PGX: patients with a non-NM metabolism for the prescribed drug

The dose used for each patient was recorded and standardized into olanzapine-equivalent doses between the different antipsychotic agents^[26]).

Clinical parameters

Clinical progression of patients was evaluated with the Positive and Negative Symptoms Scale (PANSS)^[27]. Patients were evaluated at the beginning of the study and 12 weeks after treatment. Similarly, the development of adverse events was tracked with the UKU side effects rating scale^[28] at baseline and again at 12 weeks after starting treatment. The UKU scores for different side effects were grouped into four categories: psychic, neurologic, autonomic and others. A fifth and final category, total UKU, captures the overall emergence of any side effect, as it combines the scores of all UKU categories.

Genetic profile

Whole blood samples were taken from patients at the beginning of the study. DNA was extracted with a commercial kit (QIAmp DNA mini Kit, Qiagen) following the manufacturers recommendations. Genotyping of twenty functional polymorphisms in the *ABCB1*, *CYP2C19*, *CYP1A2* and *CYP2D6* genes, including copy number variations on the *CYP2D6* gene, was performed using iPLEX® Gold chemistry and the MassARRAY platform (CEGEN-PRB2-ISCI, University of Santiago de Compostela, Spain). Table 2 summarizes the polymorphisms investigated and their predicted phenotype. The selected polymorphisms in cytochromes led to altered enzymatic activity, ranging from gene duplication related to increased protein production to loss of functional alleles. The polymorphism in *ABCB1* (3435C>T) leads to a synonymous substitution, however this change has been associated with lower expression, possibly due to linkage with a different causal polymorphism or by ribosome stalling, i.e., a less efficient translation by using a less usual

Table 2. Activity score for the studied polymorphisms

Gene	Allele designation	Allele activity score	rsID
<i>CYP1A2</i>	*1F	1.5	rs762551
<i>CYP2C19</i>	*2	0	rs12769205
	*17	1.5	rs12248560
<i>CYP2D6</i>	*3	0	rs35742686
	*4	0	rs3892097
	*5	0	gene deletion
	*6	0	rs5030655
	*7	0	rs5030867
	*8	0	rs5030865
	*9	0.5	rs5030656
	*10	0.25	rs1065852
	*11	0	rs201377835
	*17	0.5	rs28371706
	*19	0	rs72549353
	*20	0	rs72549354
	*29	0.5	rs61736512
	*31	0	rs267608319
	*41	0.5	rs28371725
	xN	2	gene duplication
<i>ABCB1</i>	3435C>T	Reduced expression	rs1045642

Activity score: 0-loss of function, 0.25-drastically reduced function, 0.5-reduced function, 1-normal function, 1.5-increased function, 2-gene duplication, rsID: accession number on the dbSNP database

codon^[29]. For *CYPs*, genotype information was converted into activity scores, according to Gaedigk *et al.*^[30] and adapted to genes other than *CYP2D6* using the same criteria. Phenotype imputation based on genotype was done according to the latest consensus article published by the Clinical Pharmacogenetics Implementation Consortium (CPIC), with the activity score for *CYP2D6**10 updated to the 2019 revision^[31,32]. The total activity score for an individual is the result of adding the scores of the two alleles they carry for that gene.

After obtaining their genetic profile, 73 of the subjects were found to be carriers of genetic combinations that would modify their ability to metabolize the drug they were prescribed. This is represented by ultrarapid (UM) for *CYP1A2* in patients on clozapine or olanzapine, UM or poor metabolizer (PM) for *CYP2C19* in clozapine users, and PMs, intermediate metabolizer (IMs) or UMs for *CYP2D6* in patients on risperidone, aripiprazole, haloperidol, pimozide or trifluoperazine.

Statistical analysis

Multivariate regression analyses of the results were performed using SPSS Statistics v23.0.0.0 (IBM). Plasma levels, change in PANSS and in UKU scores were used as dependent variables, with genetic information on the different *CYPs* (*CYP1A2*, *CYP2C19* and *CYP2D6*) and *ABCB1* being the explanatory variables. Gender, age, dose, type of antipsychotic and pharmacogenetic intervention were also included as covariates.

RESULTS

The influence of genetic factors on plasma levels was investigated using the different *CYPs* and *ABCB1* as predictors, and age, gender, dose, and the intervention arm as covariables. The full sample of 318 patients was used in all cases, but to account for the different affinities of the antipsychotics for each protein, the interaction between the antipsychotic and *CYP1A2*, *CYP2C19*, *CYP2D6* and *ABCB1* genes was used as a predictor. These interactions showed significant association with plasma levels with *P*-values < 0.001 in all cases [Table 3]. The interaction with *ABCB1* had roughly the same *P*-value as *CYP1A2* and *CYP2C19*, while

Table 3. Association of genetic factors with levels, response and side effects

	LEVELS	PANSS	T UKU	P UKU	N UKU	A UKU	O UKU
<i>CYP1A2</i>							
Model <i>P</i> -value	5.05×10^{-8}	0.39	0.03	0.01	0.19	0.72	0.08
Age	0.30	0.12	0.22	0.03	0.32	0.94	0.23
Gender	0.20	0.21	0.59	0.92	0.73	0.97	0.39
Dose	0.30	0.31	0.01	0.31	0.03	0.90	0.01
Intervention	0.63	0.96	0.37	0.08	0.85	0.82	0.94
Antipsychotic* <i>CYP1A2</i>	1.26×10^{-9}	0.40	0.06	0.01	0.58	0.10	0.75
<i>CYP2C19</i>							
Model <i>P</i> -value	3.16×10^{-8}	0.43	0.11	0.22	0.09	0.86	0.09
Age	0.45	0.07	0.27	0.03	0.25	0.84	0.25
Gender	0.13	0.22	0.43	0.81	0.68	0.90	0.39
Dose	0.18	0.36	0.01	0.34	0.02	0.91	0.01
Intervention	0.33	0.86	0.63	0.30	0.89	0.63	1
Antipsychotic* <i>CYP2C19</i>	1×10^{-9}	0.83	1	0.57	0.29	0.22	0.79
<i>CYP2D6</i>							
Model <i>P</i> -value	3.46×10^{-8}	0.5	0.08	0.07	0.13	0.75	0.09
Age	0.38	0.17	0.19	0.03	0.28	0.97	0.23
Gender	0.18	0.26	0.61	0.95	0.69	0.97	0.41
Dose	0.24	0.3	0.01	0.31	0.03	0.85	0.01
Intervention	0.56	0.90	0.42	0.1	0.91	0.74	0.87
Antipsychotic* <i>CYP2D6</i>	5.97×10^{-10}	0.49	0.29	0.12	0.25	0.11	0.99
<i>ABCB1</i>							
Model <i>P</i> -value	3.81×10^{-8}	0.44	0.66	0.17	0.12	0.84	0.07
Age	0.30	0.06	0.20	0.03	0.33	0.85	0.19
Gender	0.20	0.33	0.62	0.90	0.84	1	0.56
Dose	0.30	0.50	0.01	0.27	0.02	0.77	0.004
Intervention	0.63	0.91	0.59	0.35	0.97	0.67	0.94
Antipsychotic* <i>ABCB1</i>	1.26×10^{-9}	0.63	0.25	0.19	0.55	0.19	0.41
Affected by <i>CYP</i>							
Model <i>P</i> -value	3.46×10^{-8}	0.22	0.09	0.09	0.05	0.66	0.12
Age	0.97	0.09	0.25	0.02	0.11	0.80	0.16
Gender	0.33	0.1	0.27	0.71	0.43	0.88	0.28
Dose	0.22	0.06	0.01	0.34	0.02	0.79	0.01
Intervention	0.79	0.89	0.52	0.21	1	0.55	0.96
Antipsychotic	6.75×10^{-11}	0.73	0.44	0.08	0.11	0.08	0.96
<i>CYPs</i>	0.37	0.59	0.38	0.41	0.62	0.84	0.51

P-values for Levels: plasma levels, PANSS: change in PANSS score; T UKU: change in total UKUs, P UKU: change in psychic UKUs; N UKU: change in neurologic UKUs, A UKU: change in autonomic UKUs; O UKU: change in other UKUs

being one order of magnitude higher than *CYP2D6* (*P*-values of 1.26×10^{-9} , 1.26×10^{-9} , 1×10^{-9} and 5.97×10^{-10} for *ABCB1*, *CYP1A2*, *CYP2C19* and *CYP2D6*, respectively).

Regarding change in PANSS scores, none of the studied genetic variants showed a statistically significant association, and the models used were not statistically significant either. Regarding the change in UKU scores, *CYP1A2* models were statistically significant for total UKUs ($P = 0.03$) and psychic UKUs ($P = 0.014$). Of these models, antipsychotic**CYP1A2* resulted in a significant association with psychic UKU only ($P = 0.007$), with higher activity scores being associated with less adverse effects. The mean differences in psychic UKU at baseline minus UKU at 12 weeks was 0.67 for Activity Score (AS) = 1 (Normal Metabolizers), 2.12 for AS = 1.5 (Rapid Metabolizers) and 2.13 for AS = 2 (Ultrarapid Metabolizers). This association was further explored by comparing the psychic UKU using only the portion of the sample treated with the *CYP1A2* substrates clozapine and olanzapine, therefore removing the confounding effect of other substrates. This association did not change when the antipsychotic class was reduced to the substrates

(clozapine and olanzapine) and non-substrates of *CYP1A2*. Although antipsychotic**CYP1A2* bordered on the significance threshold for total UKU, it was statistically not significant ($P = 0.06$).

The combined effect of all *CYPs* was explored using a dichotomic variable representing whether each patient had an altered metabolism for the drug they were taking or not. In these tests, the antipsychotic was added as a covariable. However, altered metabolism was not found to be associated with plasma levels, change in PANSS or change in any of the UKU scores, although the model itself was marginally significant in relation to neurologic UKUs ($P = 0.05$).

DISCUSSION

The aim of this study was to investigate the effects of genetic variants in pharmacokinetic genes on clinical endpoints to compare their influence on the outcome of treatment. The effect of metabolism on adverse effects was observed for *CYP1A2* on psychological UKUs, but not for other clinical endpoints. No correlation between any of the investigated genetic factors with PANSS was found.

The association between *CYP1A2*, *CYP2C19*, *CYP2D6* and *ABCB1* and plasma levels, despite the very low P -values, may have explanations other than genetic variation. Unlike clinical dose data, which was transformed to olanzapine equivalents, plasma levels did not receive any standardizing transformation, and since different molecules reach different concentrations in blood, even in normal circumstances, the type of antipsychotic that can reach significance by itself, with P -values even lower than those observed for any of the antipsychotic-gene interactions, is observed at the end of Table 3. Therefore, the association observed with the interaction is most likely driven by the antipsychotic component of the interaction, which would be expected to be significant even without a real influence of the genotype. This does not rule out a true contribution of metabolism or transport on plasma levels. In fact, there have been numerous reports on the association of *CYP2D6* with plasma levels of risperidone, aripiprazole, haloperidole, zuclopenthixole, and pimozide^[3,6,10,11,13,33,34] and *CYP1A2* with olanzapine^[35], which is consistent with their metabolic pathways. For clozapine, the existing data shows a clear association with *CYP1A2* activity, including the inducer effect of smoking on plasma levels but the effect of genetic variation itself has failed to show an association in some studies^[36-38]. There are also reports on the association of *ACBC1* with plasma levels of aripiprazole and quetiapine^[39,40]. It should be noted that *CYP2D6* had the strongest association, at one order of magnitude lower than the other three genes, which could reflect a higher influence.

Regarding changes in PANSS scores, none of the *CYPs* or *ABCB1* genetic variants investigated showed any effect. In fact, no predictors of clinical efficacy were found at all in this study. *CYPs* are expected to be related more to side effects than efficacy, because most genetic variations lead to decreased metabolism, whereas the lack of efficacy is expected to be observed when metabolism is increased. Studies that have addressed the effect of metabolism on efficacy have had contradictory results. A large study by Jukic *et al.*^[3] found that both very high and very low *CYP2D6* metabolism (PMs or UMs) are predictors for treatment switch from risperidone. It should be noted that treatment switching could be due to the lack of efficacy but also, from intolerable adverse effects. van de Bilt *et al.*^[15] investigated the possibility that ultrarapid metabolism might lead to the lack of improvement, but could not confirm this hypothesis. Regarding *CYP1A2*, the studied polymorphism leads to increased expression, and thus it is reasonable to think that carriers would have a lower change in PANSS due to increased metabolism. This was observed by Czerwensky *et al.*^[35] reporting an inverse correlation between predicted *CYP1A2* activity and improvement of symptoms in patients treated with clozapine and olanzapine. Nonetheless, increased metabolism has also been described as a factor for good response. Piatkov *et al.*^[41] observed in their clozapine-treated cohort that increased metabolism through *CYP2C19* leads to a lower risk of diabetes and a higher chance of clinical improvement. *ABCB1* has also been studied regarding efficacy. Papazisis *et al.*^[17] found an

involvement of *ABCB1* in the evolution of PANSS, but only in patients with decreased *CYP2D6* activity, and also only for the rs2032582 (2677G>T/A) polymorphism in *ABCB1*, but not rs1045642 (3435C>T), which is the one used in the present study. Other authors, however, have been able to find an effect of 3435C>T on clinical improvement^[18,40]. The effect of *ABCB1* 3435C>T might be limited to specific substrates or apply only to haplotypes.

It was found that *CYP1A2* genetic variants were directly associated with changes in psychological UKU. As expected, higher *CYP1A2* activity is related to less psychological adverse effects due to the dose-dependent nature of most adverse drug reactions. The association of *CYP1A2* variants with psychological UKUs was stronger than that of age, highlighting its importance. While drug dose was associated with UKUs in most cases, this was not true for psychological adverse effects, which suggests that metabolic activity is more important than the actual dose regarding this class of adverse reactions. Local metabolism in the brain, as opposed to liver metabolism, could lead to changes in local exposure not reflected by plasma levels. *CYP1A2* is expressed in the rat brain, and has also been reported to be expressed in human brains^[42,43]. As clozapine, a *CYP1A2* substrate, is the drug that was used in more patients than any of the other agents, this study was better powered to detect differences regarding *CYP1A2*. It is possible that the lack of significant results for other genes is due to the lower relative abundance of substrates for the corresponding pathways. It is also expected that dose is related to overall side effects, but this association was not seen for autonomic or psychic UKUs. This makes the effect of *CYP1A2* on the latter particularly interesting, because it revealed a marker for adverse effects that cannot be predicted with dose. Knowing which side effects are most likely to happen can help physicians with follow-up of their patients, so that they can focus on psychic adverse effects in those patients with lower *CYP1A2* activity scores.

There are some limitations in this study that might have hampered the discovery of other associations. While the sample size of the whole study was moderate, it was not possible to divide into groups based on medications, as these groups would have been too small. Furthermore, it would have been interesting to check the possibility of drug-drug interactions, as some medications are known to be inhibitors or inducers of *CYPs* and/or *ABCB1*, so a patient can have an actual phenotype that differs from what one would predict based on genotype. However, most patients were on antipsychotic monotherapy and concomitant treatment should not have significantly affected the results. Other factors such as smoking habits and diet could not be considered due to the lack of specific information. Further studies should address these possible confounding factors.

In conclusion, there is a possible association between *CYP1A2* and psychic adverse effects, which is concordant with the known properties of clozapine. This association should be considered preliminary until it is confirmed in an independent study. None of the other studied genes showed any effect on treatment, as measured by changes in PANSS and UKU scores. These results suggest that patients with higher *CYP1A2* activity would be less likely to experience psychic adverse effects.

DECLARATIONS

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Authors' contributions

Study design: Cendrós M, Arranz MJ, Catalán R

Conducted pharmacogenetic studies: Cendrós M, Ibáñez L, Serra A, Arranz MJ

Conducted pharmacokinetic studies: Brunet M, Torra M
Performed analysis of results: Cendrós M, Arranz MJ, Catalán R
All authors contributed to the interpretation and discussion of results.

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

The research was performed in accordance with the Declaration of Helsinki and all participants gave written informed consent. AEMPS code: RCC-ANT-2013-01

Consent for publication

Not applicable.

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